Chimeric Antigen Receptors to Deplete HIV Infected Cell Reservoirs
Edward Berger (NIAID) with researchers from NIAID and NCI.

Despite the ability of highly suppressive combinations of HIV replication inhibitors to drive plasma viral loads below detection, HIV persists in the body and inevitably rebounds upon cessation of HAART. While much debate is ongoing regarding the mechanism(s)/source(s) of HIV persistence (latent reservoirs, cryptic low level replication; long-lived productively infected cells; sanctuary sites, etc.), it is generally accepted that HAART must be continued for extended periods, probably life-long. This daunting prospect, coupled with technological advances and increased understanding of HIV persistence, have reinvigorated serious consideration about possible approaches to eradicate HIV from the body, or at least to achieve a "functional cure" whereby HAART can be stopped without virus rebound. Dr. Edward Berger has co-developed (along with Dr. Ira Pastan, NCI) recombinant immunotoxins against HIV-1 Env to selectively kill infected cells as a promising approach to deplete infected cell reservoirs persisting despite HAART. However the immunogenicity of the immunotoxins (they contain a foreign domain derived from a bacterial protein) will limit treatment duration, raising concerns that HIV may re-emerge after treatment cessation, particularly in view of the persistence of latently infected cells that could eventually become activated. Hence there is a need for an alternative targeted cell killing strategy that provides more durable activity. Dr. Steven Rosenberg has pioneered adoptive cell therapy for treatment of various cancers. A particularly appealing strategy involves chimeric antigen receptors (CARs), i.e. engineered proteins containing a targeting moiety (SCFv or ligand) against a tumor-associated surface antigen linked to a TM domain and cytoplasmic signaling motifs involved in activation and antigen-stimulated proliferation of CD8 T cells. The proposed strategy is to develop CARs that mediate selective killing of HIV infected cells based on their Env surface expression. In particular, the investigators will use a novel targeting moiety developed in Dr. Berger's group called sCD4-17b that binds with extremely high affinity to Env from widely genetically diverse HIV-1 isolates. This will be converted into 2nd and 3rd generation CARs that will be transduced ex vivo into T cells using gamma retroviral vectors, and tested for expression and functional activity against HIV-infected cells. If successful, this work during the 1 year period will hopefully pave the way for clinical trials of CARs in HIV-infected patients whose viral loads are initially suppressed by HAART. When re-introduced into the patient, the CAR-transformed CD8 T cells are expected to promote efficient MHC-independent killing of infected cells. Their potential for proliferation under antigen stimulation suggests that they may persist for long periods, thus providing durable protection against re-emerging HIV and enabling infected people to remain healthy without continuous HAART.

Evaluation of Nitisinone As a Potential Treatment For Oculocutaneous Albinism
Brian Brooks (NEI) with researchers from NEI and NHGRI.

Oculocutaneous albinism (OCA) is an inherited disorder of melanin pigment production that results in decreased visual function, including legal blindness. Currently, there are no known treatments of OCA. We hypothesized that treatment with nitisinone, an inhibitor of tyrosine degradation that elevates plasma tyrosine levels, will increase the function of tyrosinase, the enzyme most often deficient in OCA in North Americans and the rate-limiting enzyme in melanin synthesis. Two mechanisms are feasible when residual tyrosinase activity is present (e.g., in OCA1B): stabilization of the enzyme, and provision of increased substrate. Preclinical studies in mice with partial loss of tyrosinase function indicate that nitisinone is capable of improving pigmentation in their skin and eyes. We wish to extend this work to include a pilot human clinical trial of nitisinone, an FDA-approved drug, in patients with OCA1B. We wish to extend our mechanistic understanding of drug action by determining whether drug efficacy can be predicted in vitro by correlating it with different missense mutations in the tyrosinase gene. Lastly, we wish to determine if a treatment effect is more generalizable to other forms of OCA.

The Multiscale Orchestration and Control of Forces in Cellular Movement
Richard Chadwick (NIDCD) with researchers from NIDCD and NHLBI.

Division, directed migration and differentiation are morphogenic cell processes that require the coordination of a number of physical events initiated by interacting cytoskeletal systems. Cells must sense their surroundings, push and pull, stiffen and soften, contract and expand. All of this must be precisely coordinated in space and time to accomplish cell processes essential for directional movement critical to development, the immune response and wound healing, and tissue regeneration. Its regulation is compromised, for example, in metastatic cancer, vascular and neurosensory disease. The relative contribution of different subcellular machines and how they are integrated to produce the forces and/or changes in stiffness required for movement has only barely been explored. Furthermore, how cytoskeletal dynamics are regulated to effect changes in physical properties of cells to mediate adaptation, specialization or disease is completely unknown.

The proposed collaboration between the Chadwick and Waterman labs will bring together emerging technologies of quantitative imaging of cytoskeletal dynamics and atomic force microscopy to develop a quantitative model of how regulated changes in the cytoskeleton mediate the physical aspects of directed cell motility. This research is at the interface between cell biology and biophysics. The Waterman lab has pioneered the use of Fluorescent Speckle Microscopy, which correlates the dynamic interaction between subcellular machines. The Chadwick lab has pioneered the development of Noncontact Frequency Modulated Atomic Force Microscopy, which measures the viscoelastic properties of tissue and cells. By combining these imaging technologies, including the sophisticated data analyses and modeling tools already developed in each lab, we will:

- map the area and height profiles of the lamellipod during the protrusion phase and under stationary conditions, with targeted molecular perturbations of specific molecular regulators of cytoskeletal polymerization or contraction
- measure local cell membrane tension gradients and cytosolic pressure gradients during the protrusion phase and under stationary conditions
- determine the gradients of viscoelastic cytoskeletal properties during the protrusion phase and under stationary conditions
- relate the above finding to subcellular dynamics obtained from fluorescent speckle microscopy under identical conditions to obtain an integrated picture of force control during the protrusion phase.

Hepatitis C: Antibodies and epitopes analyzed by phage display libraries
Stephen Feinstone (FDA) with researchers from FDA and NCI.

Hepatitis C virus (HCV) infects nearly 3 million people in the U.S. and about 170 million worldwide. Chronic HCV infections may lead to chronic liver disease, liver failure and hepatocellular carcinoma and are the leading cause for liver transplantation in the U.S.. While acute hepatitis C is often mild or even sub-clinical, the infection becomes chronic in about 75% or more of those infected. Antiviral treatment based on pegylated interferon plus ribavirin is successful in about 50% of those selected for treatment and new antiviral agents are being developed. However, there is no vaccine to prevent persistent HCV infections.

We do not understand at this time what the immune response should be that would protect exposed individuals against chronic HCV infections. Vaccines designed to induce antibodies to the envelop glycoproteins (gp)and vaccines designed to induce T lymphocyte responses to viral non-structural proteins have been proposed. In animal (chimpanzee) studies T cell vaccines have had very mixed results. Neutralizing antibody (nAb)based vaccines seem to be somewhat more protective. However, we know that in chimpanzees that recover from acute HCV infection and that resist chronic infections upon re-challenge, nAbs are generally not present or are at very low levels. Therefore, the role of nAb in providing this protection needs to be thoroughly studied.

We previously studied an antigenic region in the E2 gp that contains at least 2 epitopes termed EPI (aa 412-419) and EP II (aa 434-446). Plasma derived antibodies (from HCV infected patients or HCV infected chimpanzees) specific for EP I were able to neutralize HCV in vitro while those specific to EP II were shown to interfere with the neutralization by EP I specific antibodies. We were able to generate monoclonal antibodies (mAbs) that recognize EP II but have not yet been able to obtain neutralizing mAbs to EPI. By use of the EP II mAbs, we have further characterized this site and have shown that it actually contains a neutralization site as well as the site that binds antibodies that inhibit neutralization at EP I.

We here propose to utilize phage display technology for mining both human and chimpanzee antibody repertoires for neutralizing mAbs to EP I and EP II as well as other neutralization epitopes in the HCV envelop proteins. The proposed project is based on (i) an existing large human naïve antibody library that was previously generated from bone marrow of healthy volunteers and successfully selected against a variety of human and viral antigens and (ii)new chimpanzee immune antibody libraries that we will generate from PBMCs from a chimpanzee that had cleared an acute HCV infection and then was hyperimmunized with E1E2 to generate high levels of nAb or from bone marrow cells from a chronically infected chimpanzee that has high levels of nAb. Selected mAbs will be used to characterize HCV neutralization epitopes and perhaps be evaluated as therapeutic agents.

The Framingham DNA Methylation Initiative
Daniel Levy (NHLBI) with researchers from NHLBI, CIT, NCI, NIEHS, and NHGRI.

The risk of complex diseases is determined by the interaction of multiple genetic and environmental factors. Although genome-wide association studies (GWAS) have been highly successful in unraveling genes involved in complex diseases, common genetic variation only explains a small proportion of the heritability of these traits. It is believed that epigenetic factors, including DNA methylation, contribute greatly to the pathogenesis of complex traits. Epigenetic modifications, however, are largely unexplored in cardiovascular disease (CVD). Thus, in this multi-IC project, we seek to characterize DNA methylation in a large study sample in order to understand its contribution to CVD and other complex traits.

Specifically, we will characterize DNA methylation genome-wide in 2846 Framingham Heart Study (FHS) participants who have been comprehensively phenotyped and genotyped, and are undergoing gene expression profiling. This new resource will be deposited in dbGAP to ensure public access, and in doing so, we will expand the resources being generated by the SABRe CVD Initiative and our GWAS and exome sequencing resources. This project will generate numerous cutting-edge studies of DNA methylation – always leveraging existing resources to advance the scientific value of this repository.

We will assess the relations of differential DNA methylation across the genome to:

1. CVD and multiple other phenotypes – leveraging the extensive surveillance for events and comprehensive phenotyping in the FHS
2. RNA expression across the genome at a gene and exon level -- leveraging the genomic resources of the SABRe CVD Initiative
3. Environmental exposures (e.g. diet, smoking, obesity, hormone use) by investigating the locations, levels, and frequency of DNA methylation in relation to these traits -- leveraging the vast phenotypic resources of the FHS
4. Common genetic variation from GWAS -- leveraging the genetic resources of the FHS SHARe

The primary aims are the tip of the iceberg of what can be accomplished with a comprehensive catalogue of DNA methylation in the FHS. Because of the breadth of FHS phenotypic and genetic/genomic resources, opportunities exist for research on DNA methylation in relation to cancer, diabetes, CKD, Alzheimer's disease, osteoporosis, micronutrient intake, and thousands of other traits that have been investigated in the FHS. This project does not duplicate any ongoing FHS research activities.

We recognize that DNA methylation was a theme for the 2009 Director's Challenge, but believe it is even a more timely opportunity today, as a powerful new array from Illumina, the 450K DNA Methylation Beadchip, was released in 11/2010. It permits high throughput analysis of DNA methylation genome-wide, which will promote epigenetic discoveries and advance translational research. This project, when combined with the vast data of the FHS (including gene expression and GWAS), will further the knowledge of CVD prevention.

High resolution imaging of iPS cells
Jennifer Lippincott-Schwartz (NICHD) with researchers from NICHD and NIBIB.

Induced Pluripotent Stem (iPS) cells are promising tools for drug development, disease modeling, and tissue repair. However, better understanding of pluripotency and differentiation determinants is needed before full potential can be realized. Unfortunately, iPS cells are found infrequently (<1%) in tightly packed differentiated cell colonies and are very small (<10μm), making difficult visualization of their subcellular architectural dynamics. We propose the use of a new super-resolution imaging technique to define the dynamics of key regulators of stem cell differentiation -- mitochondrial fusion/fission and cellular contractility. Mitochondrial fission/fusion activity participates in the cell's decision to progress through the cell cycle (Mitra et al., 2009). We aim to test whether mitochondria fission/fusion activity can act synergistically with reprogramming factors in determining a cell's pluripotent state. Microenvironment stiffness has already been shown to alter the molecular composition of scaffolds connecting the cytoskeleton to the matrix in differentiated cells (Galbraith et al., 2002) and was also shown to determine stem cell lineage (Engler et al., 2006). To better understand microenvironment effects on potential iPS cells, we will monitor cell polarity, cell-cell interactions, and matrix stiffness within the colonies to determine the roles these processes may play in dedifferentiation. These analyses will be important for improving pluripotency reprogramming efficiency and ultimately for specifying the cell lineage of an iPS cell. The size and scarcity of iPS cells in the dense cell colonies present challenges for optical imaging. However, a new approach to super-resolution microscopy in thick specimens developed by Betzig and colleagues (Planchon et al., 2010) will make this possible. The new technology combines a very thin sheet of illumination with 2-photon excitation (2PE) and structured illumination to probe into biologically relevant 3D environments at speeds needed to visualize subcellular dynamics. We propose to introduce this exciting new technology to the NIH campus and further develop it in application to the study of iPS cell differentiation. One aspect of further development will be the fluorescent probes necessary for imaging these specimens. Preliminary data indicate that better 2P probes are needed to move this project forward at a reasonable pace. Thus, existing fluorescent molecules will be tested and derivatives developed for optimal 2PE imaging of iPS cells. Our overall goals are to develop methods to better identify individual iPS cells within densely packed cell colonies and to study their development and dynamics. We believe that realization of these goals will help in developing the full therapeutic potential of iPS cells and that super-resolution imaging using plane sheet microscopy is necessary to advance this work.

Molecular circuit manipulation to study higher brain function in primates
Barry Richmond (NIMH) with researchers from NIMH, NEI, and NINDS.

Molecular tools can be used to control neuronal function with more precision in anatomical target, neuronal type, and receptor subtype than has been possible before. Though developed largely in rodents, these tools can also be used in monkeys, as demonstrated in our 2004 report that injection of an antisense vector targeting the D2 receptor in monkey rhinal cortex blocked the animal's ability to associate visual stimuli with the specific reward outcome each stimulus predicted. Understanding the neural basis of cognition in humans depends heavily on understanding its neural basis in monkeys. It would be hard to exaggerate the value of being able to manipulate specific neuronal circuits in the monkey at will for understanding cognition or for eventual application of these tools to therapy in humans.

Developing molecular vectors for routine use in monkeys requires learning their spread, penetrance, and kinetics in monkeys. This work needs multidisciplinary expertise and a long-term commitment available only in a large, stable monkey research facility like ours in LN. We recently established a molecular biology lab that produces molecular vectors at high enough titers and quality for in vivo work. To study spread and penetrance, we inject locally in brain using techniques pioneered in surgical neurology/NINDS. Both AAV and lentivirus vectors with cell-specific promoters are being tested in both cortical and subcortical areas. We also started behavioral tests using systemic (chemical) activation of specifically targeted silencing tools such as ivermectin activated Cl-channels and clozapine-N-oxide-activated (CNO) G-protein-coupled receptors. We have successfully targeted tyrosine-hydroxylase-rich (TH) neurons in the substantia nigra with ivermectin activated channels, potentially providing a reversible model of Parkinson's disease. We are also studying functional efficacy of local light activation of optically gated channels (optogenetics) with a sensitive behavioral approach developed in LSR/NEI. Finally, we are improving antisense materials to achieve larger receptor knockdowns than the modest (~25%) reduction seen in our 2004 study.

Now intensive behavioral testing in monkeys is essential to evaluate the effectiveness and kinetics of reversibly silencing neuronal populations or receptors. To accomplish these goals, we need many more monkeys to assay (~15 initially), an assistant to conduct the behavioral and kinetic assays, and, given the variability of the infections, a technician to conduct anatomical and histochemical tests in mice to ensure that the vectors have sufficient biological activity before using them in monkeys. Some of these molecular tools, e.g. CNO and optogenetics, are being developed elsewhere for use in human therapeutics, so the data we collect from monkeys will provide critical input toward this important goal. The findings from our studies will thus benefit both basic and clinical neuroscience.

Volumetric Imaging of Neuronal Dynamics in the Developing Zebrafish Larvae
Hari Shroff (NIBIB) with researchers from NIBIB and NICHD.

A complete understanding of brain function would ideally include knowledge of the activity of every neuron in the brain as it processes sensory information leading to a behavioral response. The difficulties are the vast number of neurons in the brain, the challenges of in vivo recording, and the rapid rate with which information flows between them. We propose to take a first step towards in toto brain imaging by combining cutting edge microscopy techniques with newly available genetic technologies for tracking neuronal activity in zebrafish during a simple behavioral task.

Recently developed genetically encoded calcium indicators (GECI) including GCaMP3 enable neuronal activity to be monitored using fluorescence microscopy in the intact animal. If expressed ubiquitously in the nervous system, every neuron generates a fluorescent signal indicating its level of activity. Selective plane illumination microscopy (SPIM) is a technique ideally suited to monitor fluorescence signals from thousands of neurons in a large brain volume at high temporal resolution. In SPIM the sample is illuminated with a thin plane of light from the side, so that fluorescence detection occurs in a direction perpendicular to excitation. Such an experimental geometry enormously increases acquisition speed relative to point-scanning methods, as the entire imaging plane is illuminated and detected simultaneously. In addition, excitation is confined to the focal plane, drastically reducing light exposure and photodamage.

Dr. Shroff's lab has constructed a prototype SPIM which is unique on the NIH campus. Dr. Burgess's lab has generated transgenic zebrafish expressing GCaMP3 and has extensive experience establishing sensory-guided behavioral assays. Merging these two technologies would allow, for the first time, real-time volumetric imaging of neural activity in the whole zebrafish brain. However, additional technological innovations are necessary before large volume brain imaging in zebrafish is practical. Specifically we require a camera which is both exquisitely light sensitive and capable of recording at least 2000 frames per second along with optoelectronic hardware for fast focusing, sample positioning, and fluorescence signal collection.

Given the success of the technological developments outlined above, we anticipate that our collaboration will lead to the first studies on real-time, volumetric neuronal imaging in a developing organism. Such an approach could be advantageously adapted to a diverse set of applications, ranging from minimally perturbative, high resolution whole-animal imaging to high speed investigations of dynamic processes in tissues or embryos.

Genome Wide Screen for Genes that Prevent DNA Re-replication
Melvin DePamphilis (NICHD) with researchers from NICHD and the RNAi screening facility at the NIH Chemical Genomics Center.

Genome duplication in mammals normally occurs once, but only once, each time a cell divides. This restriction is circumvented on rare occasions during animal development to allow stem cells to differentiate into specialized polyploid cells that are physiologically active but that no longer proliferate. Otherwise, agents that induce mammalian cells to re-replicate their DNA before completing mitosis cause replication forks to stall and DNA strands to break. These events trigger DNA damage response pathways and eventually apoptosis. Thus, agents that selectively induce cancer cells to re-replicate their DNA without arresting the growth and proliferation of normal cells would be excellent tools in the treatment of human cancer. In fact, we recently discovered such an agent.

siRNA targeted against geminin (a protein unique to metazoan organisms that prevents DNA re-replication) induces DNA re-replication and apoptosis in many types of cancer cells, but not in normal cells (1). Although geminin was suppressed in both cancer cells and normal cells, DNA re-replication of DNA was not induced in normal cells, because normal cells have additional regulatory mechanisms that must also be blocked before DNA re-replication occurs. This discovery is the basis of a NICHD sponsored patent (2), and the development of a novel imaged based quantitative high throughput screen for small molecules that mimic the action of siRNA against geminin in human cells (3). This assay is currently under development at the NIH Chemical Genomics Center (NCGC) to identify compounds that selectively induce excess DNA replication in human cells. This assay provides a novel means for identifying molecules useful both to investigate the regulation of genome duplication and to develop potential anti-cancer therapeutics.

The object of this proposal is to identify additional genes whose action is required to restrict genome duplication in human cells to once per cell division. Based on the geminin paradigm, we anticipate that inhibition of one or more of these genes will affect cancer cells more strongly than normal cells and therefore provide additional targets for inducing DNA re replication selectively in cancer cells. Such genes will not only increase our understanding of how genome duplication is regulated during human development, but they will provide novel targets through which the compounds identified in our complementary screening might to selectively kill cancer cells. Therefore, we propose to screen for siRNAs that induce excess nuclear DNA replication either in cancer cells or in normal cells or in both. We already have adapted the assay we are currently using to screen the NCGC's small molecule library (3) to screen Qiagen's human kinome siRNA library version 4.1, and we have identified ten candidates from the more than 700 genes in this library.

RNAi screen to identify genes involved in Immunotoxin-mediated cell death
David Fitzgerald (NCI) with researchers from NCI and the RNAi screening facility at the NIH Chemical Genomics Center.

Cell death, especially in a cancer setting, is a complex process that needs considerable illumination. Here we propose a genome-wide screen using RNAi to gain understanding of immunotoxin-mediated inhibition of protein synthesis leading to cell death. Immunotoxins, antibody-toxin fusion proteins, are under investigation as novel cancer therapeutics in several Phase I/II trials in the Clinical Center, NIH1. A high rate of complete remissions has been achieved in Hairy Cell Leukemia2 and regression of small tumors has been noted in patients with mesothelioma3. However, many patients do not respond well and the basis for this apparent resistance is not currently understood. Immunotoxins bind to specific cell surface antigens that promote toxin internalization and this ultimately results in delivery of an enzymatically active fragment to the cell cytosol. In the cytosol, the toxin ADP-ribosylates EF2, leading to inhibit protein synthesis4. Usually, but not always, toxin-mediated inhibition of protein synthesis leads to cell death but via pathways which are poorly understood5. Immunotoxin activity is determined using standard cytotoxicity assays including a CellTiter-Glo Luminescent Assay5 that is readily available for high throughput screens . We propose to use this assay in cells treated with immunotoxin in combination with an siRNA Library corresponding to the whole human genome. Specifically, immunotoxins to cell surface antigens on HeLa cells will be added to produce ~ 70% decrease in viability. Candidate siRNAs will either protect cells, enhance activity or have no effect. Because apoptosis is a major focus of this study, a second screening will be conducted with a standard fluorescent caspase substrate. This will identify apoptosis-related targets. Finally, targets that enhance killing will be assayed in secondary screens of caspase activity to confirm the enhancement of immunotoxin action. Subsequent pathway analysis will focus on key genes that protect or enhance immunotoxin-mediated cytotoxicity.

Data generated will be directly applicable to both the clinical and basic sciences programs for immunotoxin development. In addition, reduction in protein synthesis is directly related to mammalian stress responses and the control of gene expression following the addition of various cytotoxic agents. Therefore, results will be broadly applicable to cell injury and repair including those following the administration of chemotherapy agents. Finally, the discovery of genes/proteins involved in immunotoxin killing could lead to new clinical protocols, where drugs modulating these pathways can be used in combination with immunotoxins to increase tumor cell killing.

Discovery of cellular aging mechanisms by RNAi-based high throughput screens
Tom Misteli (NCI) with researchers from NCI and the RNAi screening facility at the NIH Chemical Genomics Center.

The molecular mechanisms of human aging are difficult to study due to lack of suitable experimental systems. Human premature aging disorders are a powerful tool to interrogate aging mechanisms. We are using the childhood premature-aging disorder Hutchinson-Gilford Progeria Syndrome (HGPS) as a model system for the discovery of cellular aging mechanisms. HGPS is highly relevant to normal human aging since it shares several molecular, cellular and physiological hallmarks of normal aging. We will develop and apply cellular assays based on HGPS biology to identify novel mechanisms of aging by combining genome-wide RNAi screening with high-throughput/high-resolution imaging. The project will reveal novel pathways of aging and at the same time will establish a novel high-throughput approach by exploring the synergistic effects of low- and high-resolution imaging in the context of genome-wide RNAi screens.

Identification of pathways inhibiting ELG1 stabilization in response to DNA
Kyungae Myung (NHGRI) with researchers from NHGRI, NIDDK, and the RNAi screening facility at the NIH Chemical Genomics Center.

One distinctive feature of cancer cells is their persistent cell division that requires duplication of their genome. The majority of chemotherapeutic agents cause cell death by inducing DNA damage that blocks DNA replication of cancer cells. Therefore, combined approaches using conventional chemotherapeutic agents and agents that disrupt pathways protecting the genome from DNA damage during DNA replication could be an effective strategy to kill cancer cells. We recently identified a protein called ELG1 (Enhanced Level of Genome instability gene 1) in a genome-wide screen for suppressors of genomic instability. From the follow-up characterizations of ELG1's function in cell lines and mouse models, we found that ELG1 has at least two functions, (1) to unload PCNA (Proliferating Cell Nuclear Antigen) during lagging strand synthesis, which is an essential function during early mouse development, and (2) to remove ubiquitin from PCNA after translesion synthesis of DNA damage bypass, which is closely related to tumorigenesis in a heterozygous mouse model as well as several human tumors that have mutations in ELG1. At the molecular level, ELG1 is increased in response to DNA damage blocking DNA replication. When ELG1 is down-regulated by siRNA knock-down, cells die by apoptosis. Therefore, suppression of ELG1 function by reducing its protein level could sensitize cancer cells to chemotherapeutic agents. To efficiently detect the ELG1 level, we developed a cell-based assay that uses a cell line expressing ELG1 protein fused with luciferase (ELG1-luc). This assay allows changes in ELG1 protein level to be monitored by measuring luciferase activity. Thus, using this assay, we can identify pathways or chemicals that inhibit ELG1 stabilization in response to DNA damage. We are currently using the same assay to identify new small molecule probes that inhibit ELG1 stabilization (Supported by RO3, MH092164-01 roadmap grant). We wish to complement these studies with a genome-wide siRNA screen for genes that modulate ELG1 expression. Genes identified through this effort will represent potential therapeutic targets for augmenting existing chemotherapeutic agents. Furthermore, results from the RNAi screen will illuminate the cellular targets of active small molecules identified through compound screening efforts. Such relationships will potentially streamline approaches toward therapeutic strategies and further reveal functions of ELG1 in DNA repair, DNA replication, translesion synthesis for DNA damage bypass, mitosis, DNA replication, DNA recombination, and telomere maintenance.

Unraveling heterogeneity in monogenic disorders: RNAi screens for modifiers
Ellen Sidransky (NHGRI) with researchers from NHGRI and the RNAi screening facility at the NIH Chemical Genomics Center.

Untangling the web of genes contributing to the phenotypic diversity in monogenic disorders will contribute to our understanding of disease mechanisms, provide insight into complex genetic disorders and lead to new therapeutic options. Using Gaucher disease (GD) as a model, we propose genome-wide RNAi screening to probe multiple genes in well defined pathways, to assess their effect on function and to identify novel protein interactions.

GD, a monogenic recessive disorder, has well documented clinical heterogeneity and is a prototype for other lysosomal storage disorders (LSDs). In most LSDs, the disease phenotype results from lysosomal dysfunction and storage due to a specific enzymatic deficiency. All LSDs are characterized by vast phenotypic variability with limited genotype-phenotype correlation. For example, GD genotype L444P/L444P is seen in successful college students and children with autism. This heterogeneity implicates the role of modifiers and protein-protein interactions, which may include common enzyme transport and sorting pathways shared by the LSDs. Although enzyme replacement therapy successfully treats several LSDs including GD, this therapy does not enter the brain and cannot rescue severe cases. Therefore, alternative treatments are needed.

Our group, in collaboration with NCGC, used quantitative high throughput screening to identify small molecule inhibitors and activators for LSDs, and several lead compounds are being optimized as potential therapies. We view genome-wide RNAi screening as a natural expansion of this work, enabling us to probe multiple genes affecting biological processes associated with LSDs. Initially focusing on GD, then other LSDs, we will utilize already validated miniaturized enzymatic screens to identify genes that up- or down-regulate enzymatic activity when silenced. These genes may act as modifiers impacting genotype-phenotype correlations for a specific LSD, or disclose pathways shared between different LSDs.

Initially, RNAi libraries will be transfected into HeLa cells. Activity of each selected lysosomal enzyme will be assayed in cell lysates 72 hr post RNAi transfection. Candidate genes identified will be further evaluated to analyze their effect on cell viability, lysosome function and integrity, global gene expression and vesicular transport. Next, cell based assays using transfected cells and fibroblast lines from our patients with GD and other LSDs will be utilized to investigate gene function. Later,candidate genes can be knocked down in animal models. The impact of these genes will be validated using clinical parameters and samples from our thoroughly characterized patients to establish their contribution to phenotype.

The RNAi screening data from distinct LSDs will be compared to identify shared genes of interest, which may have direct relevance to lysosome biogenesis and function. Specific genes relevant to individual LSDs will be explored as potential therapeutic targets.